Neuroscience 320 (2016) 210–220

THE EFFECT OF SURGICAL AND PSYCHOLOGICAL STRESS ON LEARNING AND MEMORY FUNCTION IN AGED C57BL/6 MICE

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Abstract—Postoperative cognitive dysfunction (POCD) is an important complication following major surgery and general anesthesia in older patients. However, the etiology of POCD remains largely to be determined. It is unknown how surgical stress and psychological stress affect the postoperative learning and memory function in geriatric patients. We therefore established a pre-clinical model in aged C57BL/6 mice and aimed to investigate the effects of surgical stress and psychological stress on learning and memory function and the possible roles of the protein kinase B/mammalian target of rapamycin (AKT/mTOR) pathway. The surgical stress was induced by abdominal surgery under local anesthesia, and the psychological stress was induced by a communication box. Cognitive functions and markers of the AKT/mTOR pathway were assessed at 1, 3 and 7 days following the stress. The impairments of learning and memory function existed for up to 7 days following surgical stress and surgical stress plus psychological stress, whereas the psychological stress did not affect the cognitive function alone or combined with surgical stress. Analysis of brain tissue revealed a significant involvement of the AKT/mTOR pathway in the impairment of cognition. These data suggested that surgical stress could induce cognitive impairment in aged mice and perioperative psychological stress is not a constitutive factor of POCD. The AKT/mTOR pathway is likely involved as one of the underlying mechanisms of the development of POCD.

Key words: surgical procedures, psychological stress, learning, memory, postoperative cognitive dysfunction, AKT/mTOR signaling pathway.

INTRODUCTION

As extensive surgery in older patients becomes more common globally, postoperative cognitive dysfunction (POCD) is an important issue in perioperative care following major surgeries. At present, POCD is described as a decline in cognitive function that occurs in patients after surgery when compared to their preoperative cognitive status (Shoair et al., 2015). POCD after non-cardiac surgery has been associated with increased mortality, decreased quality of life, risk of early withdrawal from the workforce, and increased dependency (Steinmetz et al., 2009). The incidence of POCD among general or spinal anesthesia surgical patients (age $\geqslant$55 yrs.) is about 13.6% at 3 months postoperatively (Silbert et al., 2014). The etiology and pathophysiologic mechanisms of POCD in elder patients after major non-cardiac surgery are still unclear. There is emerging evidence that the peripheral inflammatory cytokines generated during surgical stress will induce neuroinflammation as well as inflammatory interactions between peripheral tissue and the central nervous system, which will harm the neurons and cause irreversible neuronal apoptosis (Liu et al., 1997; Rosczyk et al., 2008).

Psychological stress is very common during the perioperative periods. It was reported to affect 60–80% of surgical patients (Norris and Baird, 1967; Shevde and Panagopoulos, 1991), and the psychological stress before surgery could impair the wound repair process and disturb the regulation of biomarkers related to wound healing in the early postoperative period (Broadbent et al., 2003; Aberg et al., 2007; Walburn et al., 2009). But the question of whether the vulnerabilities of POCD are associated with perioperative psychological stress remains unsolved.

The protein kinase B/mammalian target of rapamycin (AKT/mTOR) pathway is an intracellular signaling pathway important in regulating neuronal development and physiology, which has been reported to have close relationships with neurologic and aging-associated diseases, such as Alzheimer and Parkinson disease (Rodgers and Theibert, 2002; Rickle et al., 2004). Studies have shown that the AKT/mTOR pathway might play a role in neuroinflammation (Tyagi et al., 2010;

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Abbreviations: AKT/mTOR, protein kinase B/mammalian target of rapamycin; ANOVA, analysis of variance; DI, discrimination index; ELISA, enzyme-linked immunosorbent assay; MWM, Morris water maze; NOR, novel object recognition; PKCα, protein kinase C alpha; POCD, postoperative cognitive dysfunction; TNF-α, tumor necrosis factor alpha.

http://dx.doi.org/10.1016/j.neuroscience.2016.02.015
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Tarassishin et al., 2011; Zhao et al., 2014). However, there are still knowledge gaps in how the AKT/mTOR pathways contribute to neuroinflammation and cognitive decline during the development of POCD.

The aim of this study was to investigate how surgical stress and psychological stress could affect postoperative cognitive function and the expression of markers of the AKT/mTOR pathway in aged C57BL/6 mice.

EXPERIMENTAL PROCEDURES

Animal

A total of 204 C57BL/6 12-month-old health female mice (weighing 25–35 g) were purchased from the company of SiBeiFu Experimental Animal Science and Technology (Beijing, China). The animals were group housed in cages of 24 × 24 × 36 cm at room temperature (22 ± 2 °C) and humidity of 50 ± 10% under standard 12–12-h light–dark cycle. Food and water were available *ad libitum* except for the times of experiments. All the mice were acclimatized for 2 weeks before the implementation of the experimental protocol.

Female mice were used in this study due to the fact that females have been shown to perform better in certain cognitive tasks, particularly novel object recognition (NOR), than males in the animal study, and stage of the estrous cycle does not affect the result of learning tasks (Sutcliffe et al., 2007).

All the protocol in this study were set in accordance with the National Institutes of Health "Guidelines for the Care and Use of Laboratory Animals" and were approved by the Animal Ethics Committee of the Chinese PLA General Hospital (Beijing, China).

Experimental design

Mice were numbered by weight and randomly assigned to three study groups (Group S: Surgical stress, Group P: Psychological stress, Group X: Psychological stress plus surgical stress), and each group was then divided into two subgroups, control and treatment (see Fig. 1).

Surgical and psychological stress. In the surgical stress group, the mice of the treatment subgroup (Group ST, $n = 34$) receive abdominal exploration surgery under local bupivacaine anesthesia, which was established by Xu et al. (2014). In brief, after the mice was gently fixed to a heating pad (37 °C), the fur of the surgical site was shaved and then cleaned with iodophor disinfection solution, and a single injection of bupivacaine (0.5%, 1 ml) was administrated to the skin and subcutaneous tissue of the abdominal area. A midline incision of the abdomen about 2–2.5 cm long was performed prior to abdominal exploration for 5 min using a sterilized cotton swabs. Afterward, the muscle and skin were stitched separately. The whole duration of the surgery was fixed at 10–12 min for each mouse. Compound lidocaine cream (25 mg prilocaine and 25 mg lidocaine, Beijing Ziguang Medication Manufacture Corporation Ltd., China) was applied every 8 h for the first and second post-operative days. No antibiotics were used. The mice in the control subgroup (Group SC, $n = 34$) received sham-surgery, only fixing, local anesthetized, and given analgesia in the same manner as the mice in group ST.

In the psychological stress group, a communication box as described by Ogawa and Kuwahara was used to produce psychological stress (Ogawa and Kuwahara, 1966). The important feature of this method is that an animal exposed to physical stress (i.e. foot shock) can induce psychological stress in another animal using an intraspecies emotional communication (Ishikawa et al., 1992). This box (50 × 30 × 30 cm) consists of a grid floor composed of stainless steel rods (Ø = 0.5 cm) placed 1.0 cm apart. The communication box was divided into 15 smaller chambers (10 × 10 × 30 cm) with transparent plastic walls. In these 15 chambers, five of the chambers in the middle were connected to a Programmable DC Power Source meter (M8831, 30V/1A, Maynuo Electronics Co., Ltd, Nanjing, China). Mice placed in this five chambers and received electric foot shock were defined as senders. The other mice placed in the other 10 chambers without electric connection were defined as responders. The responder mice were exposed to conditioned emotional stimuli such as visual, auditory, and olfactory sensations coming from the sender mice during the electric stimuli. In the treatment subgroup of the psychological stress group (Group PT, $n = 34$), sender mice received electric foot shock. In the control subgroup of the psychological stress group (Group PC, $n = 34$), sender mice did not receive electric foot shock (sham-psychological stress). The programed electrical stimuli were established according to the previously reported method with minor modifications (Katsura et al., 2002). In brief, the electric stimuli were programed as follows. First, a current of 1 mA for 15 s were applied to the mice for four times within each has a 15-s interval. Then, electric stimuli of 1.0, 1.5, 2.0, 2.3, 2.7, and 3.0 mA for 15 s with 15 s intervals were applied to animals under the same condition as the first step. The electric stimuli program was implemented three times in the morning between 9:00 am to 12:00 am and in the afternoon between 2:00 pm to 5:00 pm for 15 consecutive days. Sterile water sprays were applied on the rods before placing the mice into the chamber to ensure an intact electric conduction.

In the combine stress (psychological stress plus surgical stress) group, the treatment subgroup (Group XT, $n = 34$) received 15 consecutive days of psychological stress followed by abdominal exploration surgery within 24 h after the last electric stimuli. Whereas the control subgroup (Group XC, $n = 34$) received 15 consecutive days of sham-psychological stress followed by sham-surgery within 24 h after the last sham-electric stimuli.

Open field test. To determine whether the future behavioral changes were attributable to changes in spontaneous locomotor activity, anxiety, and adaptivity to the given task environment, open field tests were performed 2 h before the first NOR test. A number of 16 mice in each subgroup (96 mice in total) were randomly selected and subjected to behavior test in this study.

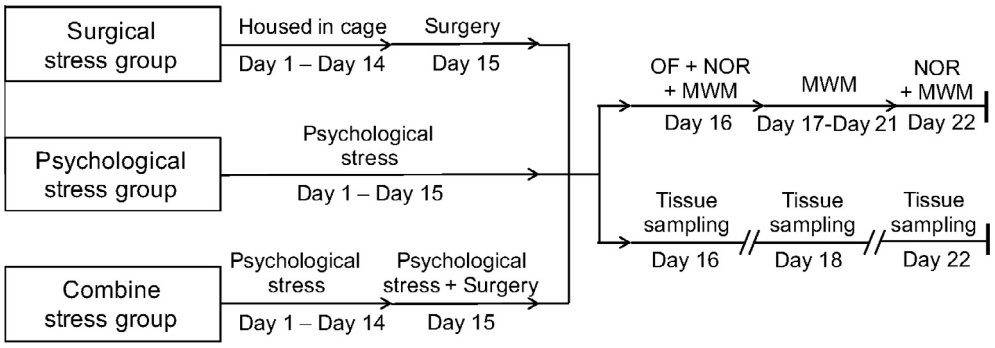

Fig. 1. Experimental design. There were three study groups: surgical stress group, psychological stress group, and combine stress group (surgical stress plus psychological stress). In each study group, there were two subgroups, treatment and control. The preparation of the animal models lasted for 15 days. Then, sixteen mice from each subgroup were randomly selected to enter behavior tests, and the other 18 mice entered tissue sampling.

The open field test was performed as described in the previous study (Chaviaras et al., 2010). The open field apparatus consists of a 50 × 50-cm square floor surrounded by 30 cm high wall, which was divided into three concentric square shape zones representing the close, medium, and far distance to the central of the field. The test was performed under a dark light of 50 l×. Mice were placed in the center of the open field and allowed to explore the area freely for 5 min. Their activities were recorded by an overhead video camera. The moving distance as well as the time staying in each zone of the open field was analyzed using animal behavior tracking system (Smart, San Diego Instruments, San Diego, CA, USA). The open field apparatus was thoroughly cleaned after the test of each mouse.

NOR task. The NOR task was used to evaluate cognition, especially recognition memory. The NOR task protocol was performed according to a previous study with little modifications (Arnt et al., 2010). In brief, the mice were habituated to the NOR chamber (30 × 30 × 20 cm) for 10 min before the start of testing. Then, two same identical objects were placed and fixed in the middle of the NOR chamber with a 10-cm interval. The mice were exposed to these two objects for 3 min. After that, the mice were returned to their home cage for an inter-trial interval of 15 min, the entire chamber was cleaned, both objects removed and one replaced with an identical familiar copy and one with a novel object. Following the interval, mice were returned to explore the familiar and novel objects in the NOR chamber for a 3-min retention trial. The chamber was cleaned with a 70% ethanol spray and water between subjects. The object exploration is defined as mice sniffing, licking or touching the objects with forepaws, and the exploration time of each object was recorded manually with two stopwatches. The discrimination index (DI) was calculated after each trial (DI = [the difference in exploration time between the novel and familiar objects]/[the total exploration time of both objects in the retention trial]). The NOR tests were performed at 24 h and 7 days after the last treatment in each group.

Morris water maze (MWM). MWM testing was performed to assess spatial learning, spatial memory and cognitive flexibility (Morris et al., 1982). The mice will be placed in a large circular pool (diameter 140 cm) filled with white non-toxic paint and water of 26 ± 1 °C to a depth of 25 cm. The circular pool was surrounded by external visual cues, and the maze was divided into four quadrants. In one quadrant (target quadrant), a transparent round platform of 9 cm of diameter was placed 0.5–1 cm below the horizontal surface.

The MWM training started 24 h after the last treatment in each group. All the mice were trained for seven consecutive days with three massed trials administered each day and the platform remained in the same location. Mice were placed on the platform for 30 s ahead of the start of each training session. During the three trials, mice were randomly placed in the water in one of the three quadrants without the platform. Mice were allowed to swim freely for 60 s or until the platform was reached. Each trial stopped 5 s after the mice stood on the platform. If the mice did not find the platform within 60 s, a guide stick was used to help the mice to find the platform. After completion of the trials the mice were towel dried and placed back to their home cage under a heat lamp for 10 min. After 7 days of training, the hidden platform was removed form the circular pool, and the mice were tested in the water maze for 60 s. Time spend in the target quadrant was taken as measure for spatial memory.

Swimming speed, latency to the platform and swimming distance were monitored by a video camera fixed to the ceiling directly above the middle of circular pool, and the camera was used in conjunction with a computerized animal behavior tracking system (Smart, San Diego Instruments, San Diego, CA, USA).

Tissue sampling

After 1 day, 3 days and 7 days the animals were sacrificed by cervical decapitation under deep sevoflurane anesthesia. Blood samples were taken directly from the heart following thoracotomy, and transcardial perfusion was performed with ice-cold standard phosphate-buffered saline. Then, the brain was immediately removed and washed in cold saline. The cortex and hippocampus were dissected and collected carefully in a sterile tube prior to being snap-frozen in

liquid nitrogen. The blood samples were centrifuged for 10 min at 2600 rpm. The supernatants were collected and stored at −80 °C until required for enzyme-linked immunosorbent assay (ELISA) analysis.

ELISA

Plasma tumor necrosis factor-alpha (TNF-α) concentrations were determined using the mice TNF-α ELISA-kit (Dakewe Bio-technology, Shenzhen, China) according to the manufacturer's instruction.

Immunoblotting

Samples from the different groups were homogenized on ice using RIPA buffer (1% Triton X-100/1% sodium deoxycholate/0.1% $NaDodSO_4$/15/150 mM NaCl/10 mM Tris·HCl, pH 7.2) plus protease inhibitors (1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). The lysates were collected, centrifuged at 13,200 rpm for 15 min, and then quantified for protein concentrations by a BCA protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA). Samples were separated on gradient sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and transferred onto a polyvinylidene difluoride membrane (PVDF, Whatman, England), which is then blocked with 5% non-fat milk. Afterward, the membranes were incubated with primary antibodies overnight. The primary antibodies used in this study were anti-AKT (Cell Signaling Technologies; #9272), anti-p-AKT (Cell Signaling Technologies; #4060), anti-mTOR (Cell Signaling Technologies; #2972), anti-p-mTOR (Cell Signaling Technologies; #2971), anti-PKCα (Santa Cruz Biotechnology, sc-8393) and anti-p-PKCα (Santa Cruz Biotechnology, sc-12356). After three washes with TBST buffer, the membranes were incubated with goat anti-mouse-HRP and goat anti-rabbit-HRP for 30 min each. The images were digitized from the membrane and the band intensity was quantified using image J analysis software (developed by the National Institutes of Health; available at: http://rsb.info.nih.gov/ij/).

Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. Repeated measures analyses of variance (ANOVAs) were used to analyze the difference of escape latency between each group in the MWM test. Other results were subjected to a one-way ANOVA followed by *post-hoc* LSD test was used to determine statistical differences between the experiment groups. p values less than 0.05 were considered statistically significant.

RESULTS

Exploratory behavior

Although the total moving distance of mice in subgroup XT seems shorter than the other subgroups in the open field, there was no significant difference among all subgroups ($F = 0.887$, $p = 0.493$). Time spent in the far distance zone, which was perceived to be the safest for the mice, appeared to be longer for the subgroups that had psychological stress (Group PT an XT), although the difference between subgroups did not reach statistical significance ($F = 2.097$, $p = 0.073$) (Fig. 2).

Learning and memory

The learning and memory of the mice were evaluated using two methods, NOR task and MWM test.

In the first NOR task at 24 h after the last experimental treatment, the exploration time for the novel object was significantly longer in each subgroup ($p < 0.05$). The DI did not have statistically significant differences between subgroups ($F = 1.380$, $p = 0.239$), although on average the index seemed decreased in group ST and group XT.

In the second NOR task performed on the seventh day after the last experimental treatment, the mice that underwent abdominal exploration surgery failed to discriminate between a familiar and a novel object in the second testing session, while the mice in the

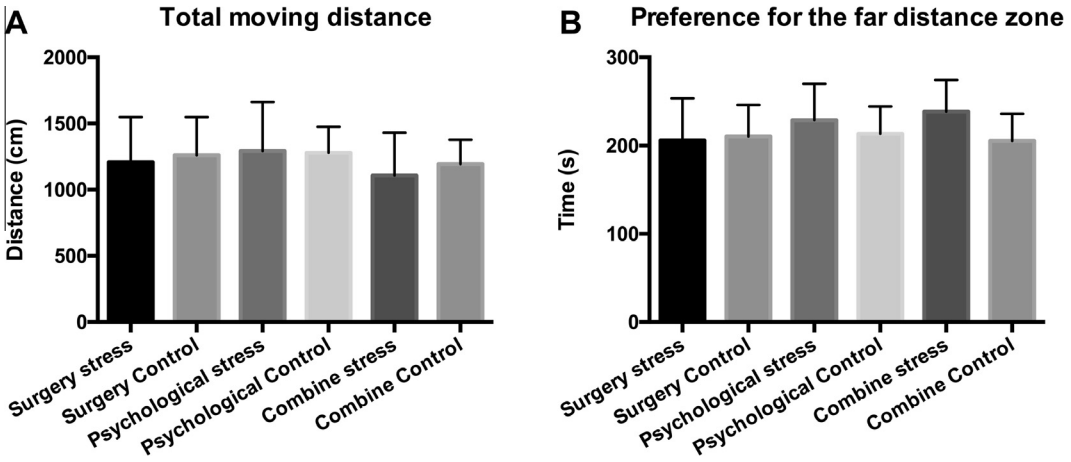

Fig. 2. Assessment of the effects of surgical stress, psychological stress and combine stress on aged mice in the open field test. At 24 h after the last treatment in each subgroup, the total moving distance and preference for the far distance zone did not differ from each subgroup ($p > 0.05$).

psychological stress group (group PT) and all the control groups (group SC, group PC and group XC) clearly directed exploration toward the novel object. Similar results were obtained when results were expressed as DI in Fig. 3.

In the MWM test, both the swimming speed and distance during the first trial were not significantly different between the subgroups ($p > 0.05$). For latency to find the hidden platform, a repeated-measures ANOVA revealed a significant effect of a 7-day training session ($F = 121.1$, $p < 0.001$), indicating all mice were able to learn where the platform was located. However, the learning curve differed significantly between groups ($F = 2.884$, $p < 0.001$) with group ST and group XT showing impaired learning function compared to their own control subgroup SC ($p < 0.001$) as well as group XC ($p = 0.001$). Group PT did not differ from its own control group PC ($p = 0.927$), but did differ from group ST ($p < 0.001$) and group XT ($p < 0.001$). No significant difference was found between group ST and group XT ($p = 0.682$), or between the three control groups (group SC, group PC and group XC, $p > 0.95$). After 7 days of MWM training, the platform was removed and the mice were tested for spatial memory. Although group ST and group XT had less time spent in the target quadrant compared to the other groups, the ANOVA did not reveal a significant effect of groups ($F = 2.097$, $p = 0.073$) (Fig. 4).

Plasma TNF-α

Plasma TNF-α increased significantly at day 1 after the last experimental treatment in group ST ($p = 0.035$) and group XT ($p = 0.013$), when compared to its own control. At day 3, there was no significant difference in either group ST compared to group SC ($p = 0.055$) or group XT compared to group XC ($p = 0.145$), although there was a trend of higher plasma TNF-α level in the treatment subgroups which underwent surgical procedures. At day 7, no statistical difference in plasma TNF-α level was found between each subgroup (Fig. 5).

Expression of phosphorylated-AKT

Our experiment found that the expression of phosphorylated-AKT protein level in the hippocampus was remarkably suppressed and the AKT expression was unchanged in the groups that underwent abdominal surgery (Group ST and group XT) at both day 1 and day 3. However, in the cortex, the phosphorylated-AKT expression was elevated at day 1 and then significantly suppressed at day 3 in group ST and group XT (Fig. 6).

Expression of phosphorylated-mTOR

The phosphorylated-mTOR in the hippocampus was slightly decreased in group ST and group XT at day 3 compared with that of their own control groups. Similar

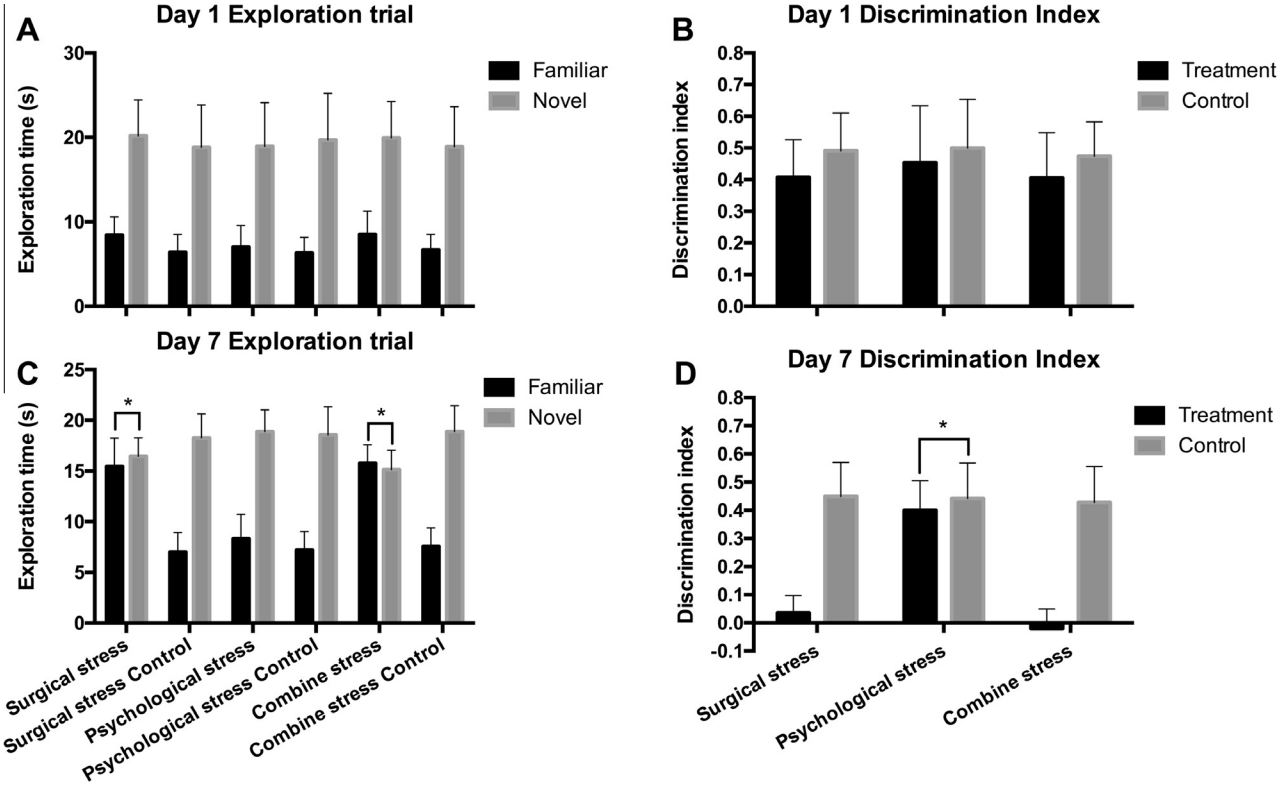

Fig. 3. The effects of surgical stress, psychological stress and combine stress on discrimination index and mean exploration time of familiar and novel objects at day 1 and day 7 after the last treatment. Each vertical bar represents the mean ± SD ($n = 16$ in each subgroup). *$p > 0.05$. (A) The exploration time of novel object was significantly higher in each group at day 1. (B) There were no significant differences in discrimination index within each study group at day 1. (C) There was no significant difference in exploration time of novel object compared to familiar object in the surgical stress and combine stress subgroups. (D) Only the difference of discrimination index in the subgroups of the psychological stress group was not statistically different at day 7.

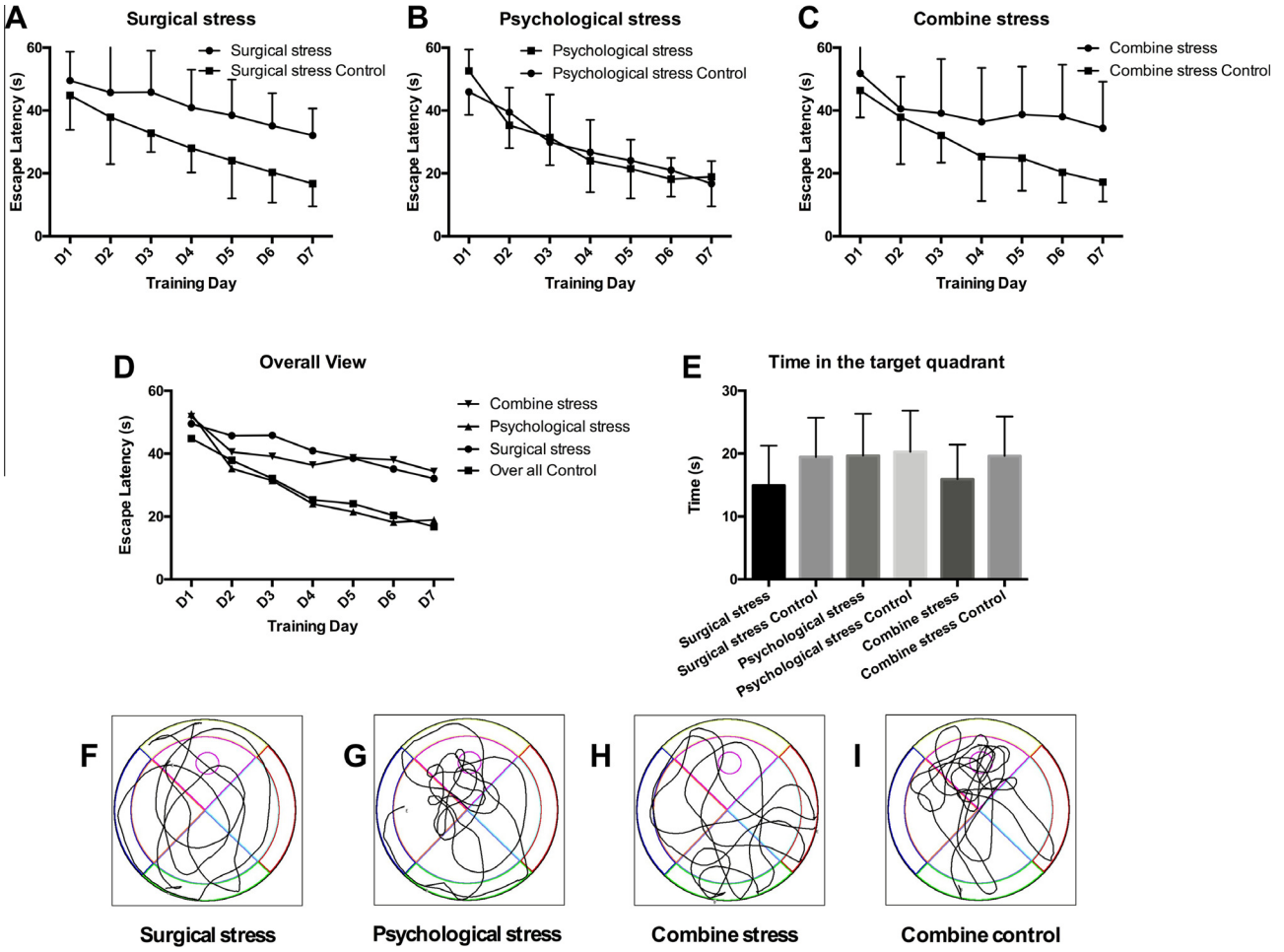

Fig. 4. Spatial learning performance in the Morris water maze. Average escape latency (s) is shown for the 7 training sessions in the maze ($n = 16$ in each subgroup). (A) Surgical stress group; (B) Psychological stress group; (C) Combine stress group; (D) the overall view of escape latency in 3 stress treatment subgroups and the combination of 3 stress control subgroups. (E) No difference was found in the time spend in target quadrant within each group at day 7. Trajectories of mice from each experimental group in the Morris water maze tests after the hidden platform was removed at day 7 were shown in (F–I).

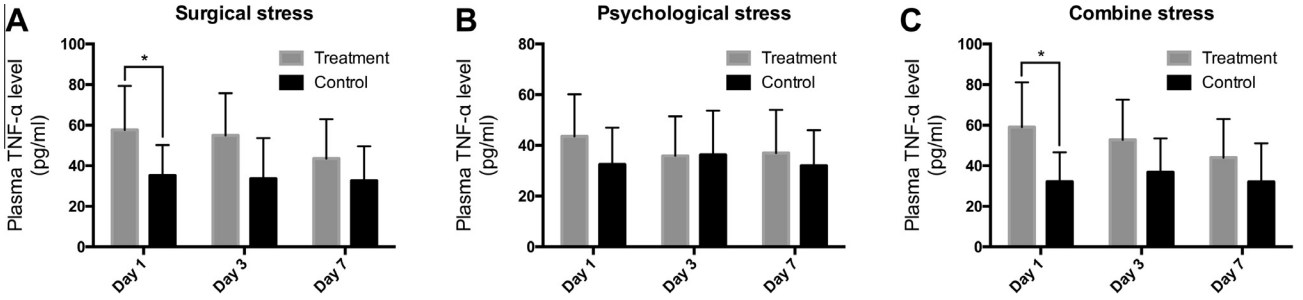

Fig. 5. Plasma TNF-α concentrations (pg/ml) at day 1, 3 and 7 after the surgical stress, psychological stress and combine stress. Each vertical bar represents the mean ± SD ($n = 6$ in each subgroup). $^{*}p < 0.05$. At day 1 of surgical stress group (A) and combine stress group (C), plasma TNF-α concentrations were significantly elevated in its treatment group compared to control group.

results were also found in the cortex: the phosphorylated-mTOR level in both group ST and group XT was significantly reduced at day 3. At day 7, the phosphorylated-mTOR level in the cortex was still lower than group XC, whereas no difference was found in the hippocampus (Fig. 7).

Expression of phosphorylated-PKCα

The changes of the expression of phosphorylated-PKCα were similar in both the hippocampus and the cortex. The phosphorylated-PKCα level in both the hippocampus and the cortex were elevated in all the experimental treatment groups at day 1 when compared

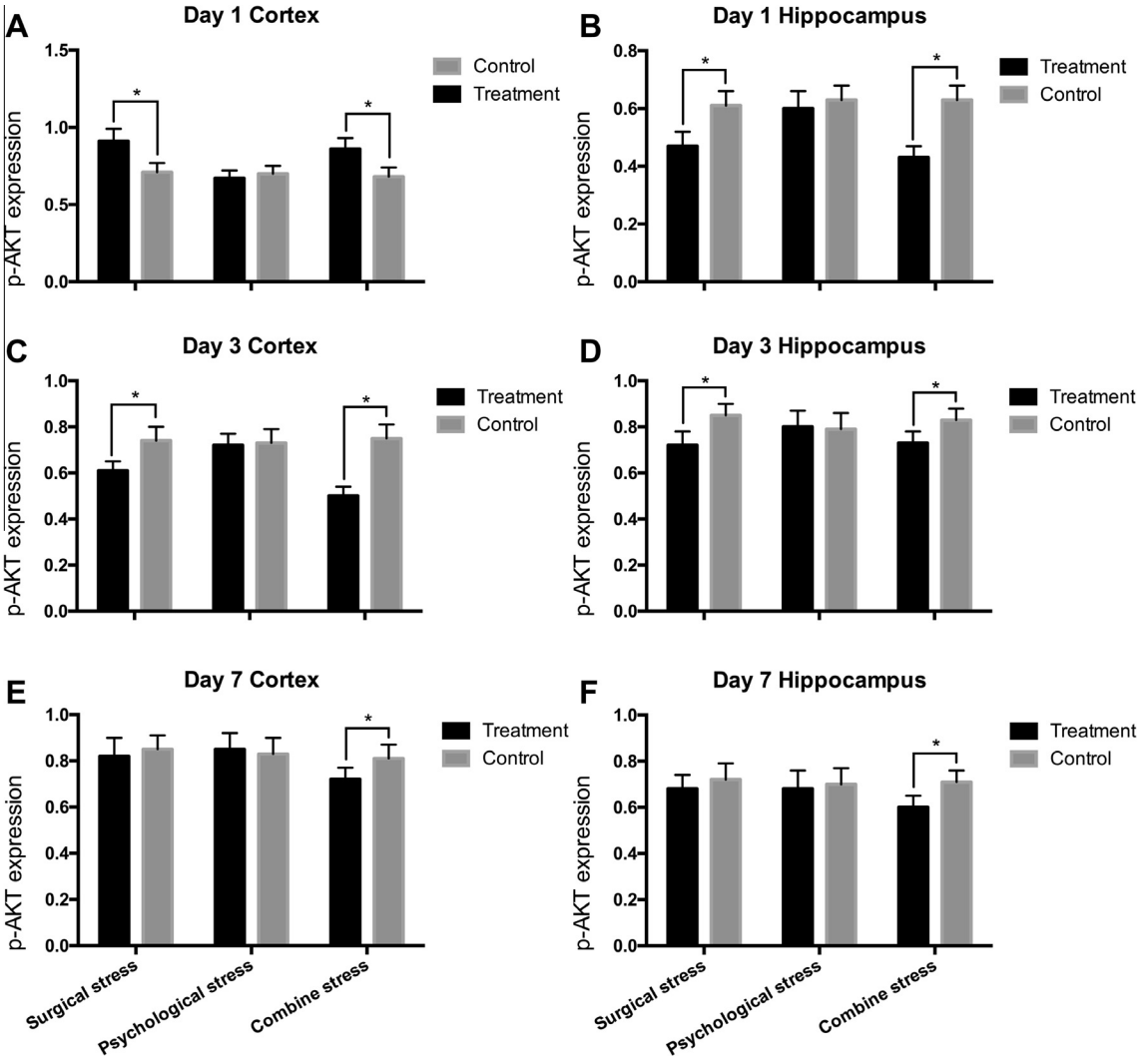

Fig. 6. The protein expression of phosphorylated-AKT at day 1, 3 and 7 in both the cortex and hippocampus. Each vertical bar represents the ratio between phosphorylated-AKT/AKT (mean ± SD, $n = 6$ in each subgroup). $^{*}p < 0.05$. At day 1, the phosphorylated-AKT expression was significantly increased in the cortex (A) and decreased in the hippocampus (B) in the treatment subgroup of the surgical stress group and combine stress group. At day 3, the phosphorylated-AKT expression was significantly decreased in both the cortex (C) and hippocampus (D). At day 7, the phosphorylated-AKT expression was still lower only in the cortex (E) and hippocampus (F) of treatment subgroup in combine stress than its control. The expression of phosphorylated-AKT did not change from day 1 to day 7 in both the cortex and hippocampus.

to their control groups. The phosphorylated-PKCα level then returned to normal at day 3 and day 7 (Fig. 8).

DISCUSSION

Despite a rapid growing awareness of the importance of POCD as a major complication in aged patients after anesthesia and surgery, specific reasons for this kind of cognitive impairment remain unclear. Many studies aimed to determine the pathogenesis of POCD in surgical patients. However, evidence showed no significant difference in the incidence of POCD between general anesthesia, abdominal surgery or general anesthesia plus abdominal surgery, and no study had ever reported the effect of psychological or psychological stress followed by surgical stress on cognitive function. We therefore established a pre-clinical model in aged C57BL/6 mice and aimed to explore the surgical stress and psychological stress on learning and memory function and the possible roles of the AKT/mTOR pathway. We found that peripheral abdominal surgical stress caused suppression of phosphorylated-AKT level, suppression of phosphorylated-mTOR level, increased levels of phosphorylated-PKC α in the brain, and increased TNF-α in the plasma. Cognitive impairment in aged mice was caused by abdominal surgical stress but not psychological stress. Psychological stress alone did not impair cognitive function in aged mice, but could alter the level of phosphorylated-PKC α in the hippocampus and the cortex. The cognitive impairment of the aged mice seems not be deteriorated after the combination of surgical and psychological stress.

In the present study, we found that shortly after the peripheral abdominal surgery with local anesthesia, the mice started to develop cognitive dysfunction with impaired learning and memory ability in the MWM and

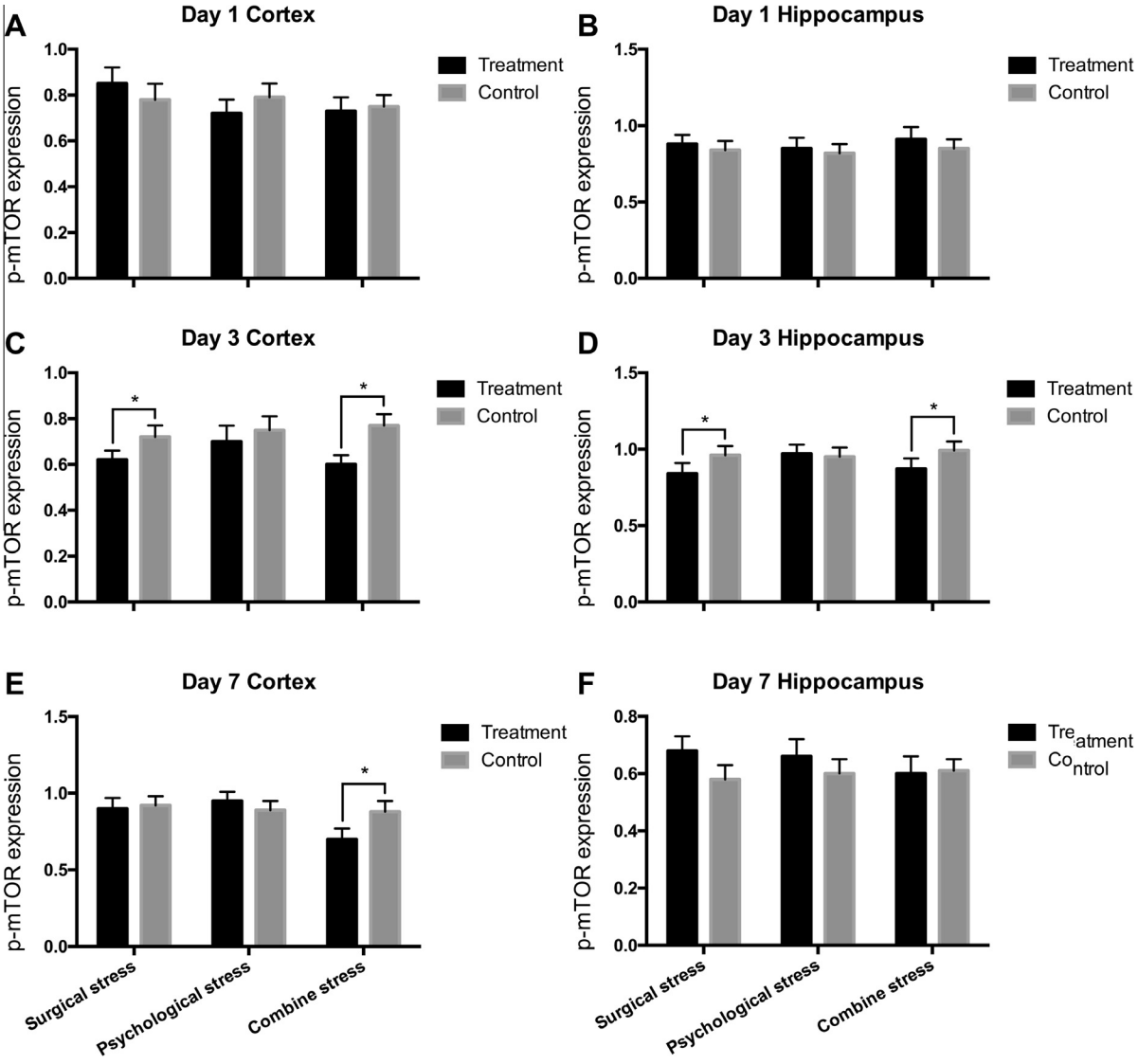

Fig. 7. The protein expression of phosphorylated-mTOR at day 1, 3 and 7 in both the cortex and hippocampus. Each vertical bar represents the ratio between phosphorylated-mTOR/mTOR (mean ± SD, $n = 6$ in each subgroup). $^{*}p < 0.05$. At day 1, no change of the expression of phosphorylated-mTOR was found in both the cortex (A) and hippocampus (B) of all the groups. At day 3, the expression of phosphorylated-mTOR was significantly suppressed in both the cortex (C) and hippocampus (D) of surgical stress treatment subgroup and combine stress treatment subgroup. At day 7, the expression of phosphorylated-mTOR was still suppressed in the cortex (E) of the combine stress treatment subgroup, while no significant suppression was found in the hippocampus (F) of all the groups.

NOR task. The impairment of cognition lasted for at least 7 days after the surgery. The mice with surgical procedures had an average escape latency and DI of 90% longer and 40% lower, respectively, when compared to the control and psychological stress mice. The effect of surgical stress on cognition function is in accordance with the result of the studies from Xu et al. (2014). They demonstrated that peripheral surgery was able to induce cognitive impairment independent of general anesthesia in 18-month-old mice, and the impairment of cognition was caused by the brain Aβ accumulation. In another study, Hovens et al. found that hippocampal dependent learning and memory were vulnerable to impairment as a consequence of abdominal-surgery-induced neuroinflammation, and the changes in exploratory behavior were correlated to plasma IL-6 concentrations at 24 h after surgery (Hovens et al., 2014). Moreover, Rosczyk et al. and Fidalgo et al. reported that peripheral surgery could disrupt hippocampal-dependent learning and memory due to the increase of IL-1β level in the brain of aged animals (Rosczyk et al., 2008; Fidalgo et al., 2011). Thus, we can infer that the impairment of cognitive function in aged mice after peripheral surgery may be due to a series of complex biochemical changes in the brain, which are related to both neuroinflammation and neuron apoptosis.

However, in those mice receiving psychological stress from the communication box, no signs of cognitive impairment were identified using the MWM and NOR task. Only a slight increase, not statistically significant, in the preference for the far distance zone in the open field test was observed in the psychological stress

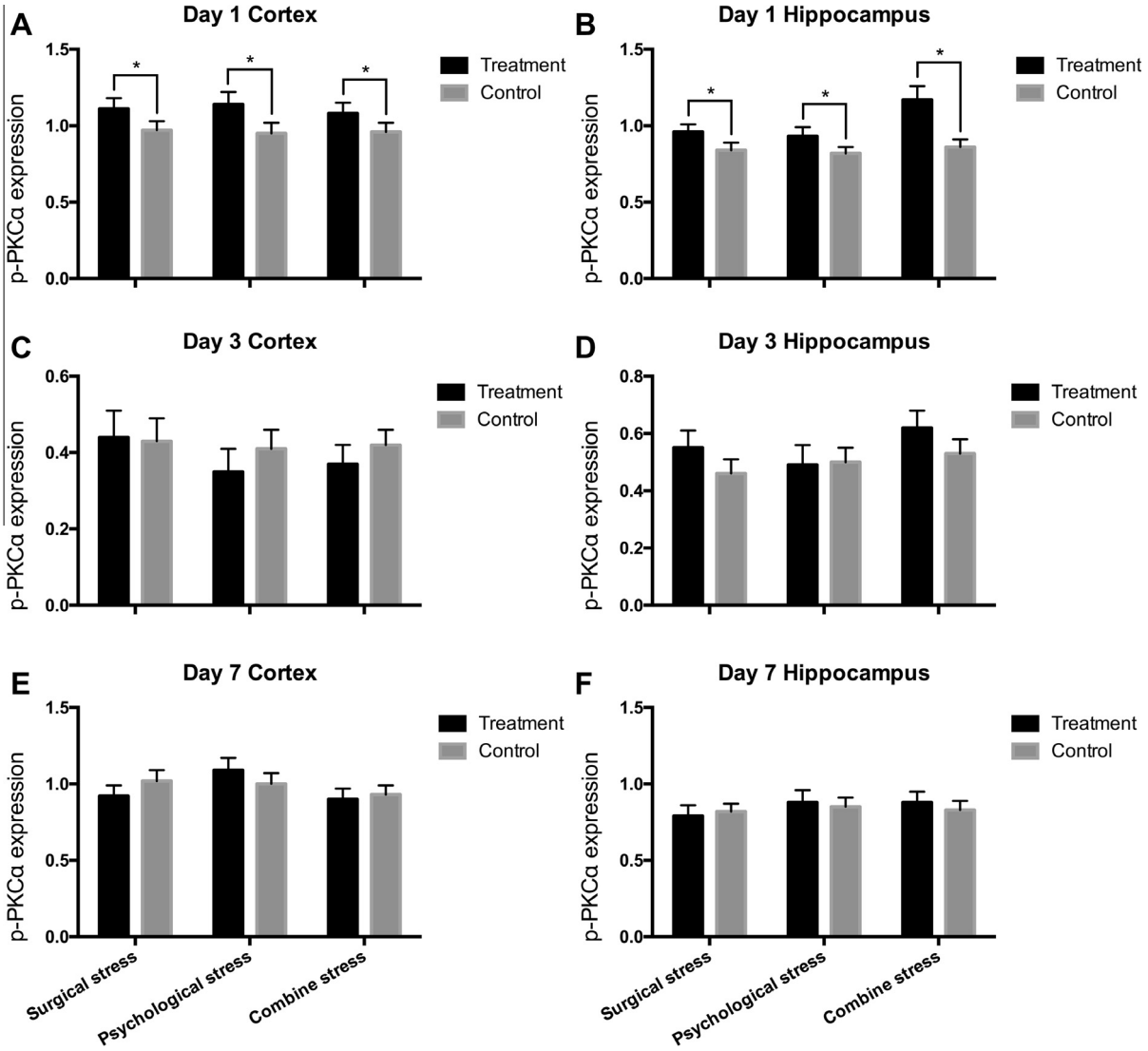

Fig. 8. The protein expression of phosphorylated-PKCα at day 1, 3 and 7 in both the cortex and hippocampus. Each vertical bar represents the ratio between phosphorylated-PKCα/PKCα (mean ± SD, $n = 6$ in each subgroup). $^{*}p < 0.05$. At day 1, the expression of phosphorylated-PKCα in both the cortex (A) and hippocampus (B) of all the treatment subgroups were elevated. The phosphorylated-PKCα level in both the cortex and hippocampus returned to normal in each group at day 3. No changes were found between day 3 and day 7.

model mice. The psychological stress model, induced by the communication box, did not alter the cognitive status of surgical stress mice. Our study is the first to test the learning and memory function instead of corticosterone levels in rodents following psychological stress induced by a communication box (Ishikawa et al., 1992; Endo et al., 2001). The possible explanation for this phenomenon is that (I) although aged mice are more sensitive to both the cognitive and inflammatory effects of mild stress than are adult mice (Buchanan et al., 2008), the intensity of psychological stress in this study may not reach the threshold of altering cognitive function in aged mice (Lupien and McEwen, 1997); (II) female mice have a quicker ability to recover from cognitive impairment after chronic stress (Conrad et al., 2003).

To investigate the potential mechanisms of how surgical and psychological stress affect the learning and memory, expressed as the results of MWM and NOR task, we focused our biochemical analyses on the AKT/mTOR pathway in the hippocampus and the cortex. We chose to analyze the protein expression in the hippocampus and the cerebral cortex because they govern cognitive and behavioral function in the mammalian brain (Atallah et al., 2004). Many recent reports observed that the change of protein expression in the brain led to change in cognitive and behavioral function (Rosczyk et al., 2008; Wan et al., 2010; Xu et al., 2014). In our study, various degrees of decreases in phosphorylation of AKT and mTOR in the hippocampus were observed at day 1 and day 3 in both of the groups undergoing abdominal surgery. These results agree with the previous experimental study performed by Gong et al., which suggested that down-regulation of phosphorylated-AKT may contribute to the impairments

of learning and memory functions in stroked mice (Gong et al., 2012). While in the cortex, we observed a short-term elevation of phosphorylated-AKT after abdominal surgery, which may be explained by the loss of temporal regulation or aberrant control of AKT in the neurons (Griffin et al., 2005).

The suppression of the expression of both phosphorylated-AKT and phosphorylated-mTOR in the hippocampus is concomitant with a cognitive decline in mice underwent abdominal surgery, measured by behavior tests. The consistency between the behavior and biochemical analysis demonstrated that the impairment of cognitive function of aged mice after abdominal surgery, in the absence of general anesthetics, is related to the suppression of the AKT/mTOR pathway. As reported previously, the activation of AKT through the phosphorylation of several effectors in the hippocampus following the stimulating of injury factors is mainly considered in the context of limiting apoptosis of the neurons (Wang et al., 2014), and the mTOR, which is the downstream substrate of PKB/AKT signaling, was also reported to regulate many major cellular process and pathological conditions including neurodegeneration (neuronal apoptosis) (Laplante and Sabatini, 2012). Regulated AKT/mTOR signaling is critical for synaptic plasticity mechanisms, and the inhibition of AKT/mTOR in neurons might impair synaptic protein synthesis needed for normal learning and memory (Graber et al., 2013). Therefore, from a neuron-centric view, the continuous suppression of the AKT/mTOR pathway at day 1 and day 3 after abdominal surgery might be expected to have a certain extent of neuron dysfunction, apoptosis and even death, which led to the cognitive impairment observed by NOR test at day 7.

We identified several weaknesses in our study. First, all the mice were observed and tested only for 7 days after surgery; the long-term effect of peripheral abdominal surgery on cognitive function remains unknown. Second, no method of rescuing cognitive impairments (i.e. using corresponding drugs or antibodies to reduce the neuron apoptosis) was tested in this study. Third, only female mice were used in this experimental study. Finally, animal models used in this study cannot completely reproduce the clinical situation of POCD and its complexity.

CONCLUSION

The results of this study indicate that surgical stress (under local anesthesia) played an important role in postoperative cognitive impairments in aged mice and that the AKT/mTOR pathway is likely a part of the underlying mechanisms of this process. The psychological stress, generated by the communication box, is not a constitutive factor of post-operative cognitive impairment following abdominal surgery in aged mice. Further studies are needed to comprehensively examine the underlying mechanisms of neuronal apoptosis followed by surgical stress and the potential therapeutic methods of rescuing the cell death and cognitive impairment.

AUTHOR CONTRIBUTIONS

Changsheng Zhang designed the experiment, performed MWM, ELISA, Western-blotting measurement and drafted the manuscript. Changtian Li performed open field test, NOR, ELISA, Western-blotting measurement and drafted the manuscript. Zhipeng Xu designed the experiment and prepared the animal models. Siqi Zhao performed open field test, MWM, and NOR. Peng Li prepared the animal models and contributed to data analysis and interpretation. Jiangbei Cao contributed to data interpretation and manuscript editing. Weidong Mi, designed the experiment, edited the manuscript and took overall responsibility for the work.

CONFLICT OF INTEREST

None.

FUNDING

This work was supported by the National Natural Science Foundation of China (Nos. 81371204, 81471119).

Acknowledgments—The authors thank Jun Ma, Ph.D. from the Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, for valuable suggestions and assistance in the sample analysis.

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(Accepted 5 February 2016)
(Available online 9 February 2016)